Effect of pre-treatment with dichloroacetic or trichloroacetic acid in drinking water on the pharmacokinetics of a subsequent challenge dose in B6C3F1 mice

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Abstract

Dichloroacetate (DCA) and trichloroacetate (TCA) are prominent by-products of chlorination of drinking water. Both chemicals have been shown to be hepatic carcinogens in mice. Prior work has demonstrated that DCA inhibits its own metabolism in rats and humans. This study focuses on the effect of prior administration of DCA or TCA in drinking water on the pharmacokinetics of a subsequent challenge dose of DCA or TCA in male B6C3F1 mice. Mice were provided with DCA or TCA in their drinking water at 2 g/l for 14 days and then challenged with a 100 mg/kg i.v. (non-labeled) or gavage (^{14}C-labeled) dose of DCA or TCA. The challenge dose was administered after 16 h fasting and removal of the haloacetate pre-treatment. The haloacetate blood concentration–time profile and the disposition of ^{14}C were characterized and compared with controls. The effect of pre-treatment on the in vitro metabolism of DCA in hepatic S9 was also evaluated. Pre-treatment with DCA caused a significant increase in the blood concentration–time profiles of the challenge dose of DCA. No effect on the blood concentration–time profile of DCA was observed after pre-treatment

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with TCA. Pre-treatment with TCA had no effect on subsequent doses of DCA. Pre-treatment with DCA did not have a significant effect on the formation of $^{14}CO_2$ from radiolabeled DCA. In vitro experiments with liver S9 from DCA-pre-treated mice demonstrated that DCA inhibits it own metabolism. These results indicate that DCA metabolism in mice is also susceptible to inhibition by prior treatment with DCA, however the impact on clearance is less marked in mice than in F344 rats. In contrast, the metabolism and pharmacokinetics of TCA is not affected by pre-treatment with either DCA or TCA. © Published by 1999 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Pre-treatment; Dichloroacetic or trichloroacetic acid; Drinking water

1. Introduction

Halogenated acetic acids or haloacetates, are a major class of by-products found in drinking water treated with chlorine. Typically, total haloacetate levels are second only to the trihalomethanes, but in some locations the haloacetates may exceed trihalomethane concentrations [1]. Dichloroacetate (DCA) and trichloroacetate (TCA) are the most commonly identified haloacetates in finished drinking water. Dichloroacetate and TCA are also metabolises of trichloroethylene (TRI), a common soil and ground contaminant [2]. The fact that DCA, TCA and TRI are all hepatocarcinogens in mice [3–9] has prompted our investigation on the metabolism and disposition of challenge doses of DCA or TCA after prior exposures.

It has been reported that prior exposure to DCA in humans increases the elimination half-life of subsequent oral or i.v. doses [10]. The metabolism of DCA is now known to occur primarily in the liver [11]. Subsequent to these studies, we demonstrated that delayed elimination of DCA in male F344 rats previously exposed to DCA, was caused by the auto-inhibition of a gluta-thione dependent pathway in the liver [12]. These results were later corroborated by James et al. [13] with DCA-pre-treatment in male Harlan Sprague–Dawley rats.

Prior studies [14–16] of metabolism and pharmacokinetics of DCA and TCA in mice are mainly based on doses to naive animals. The effect of prior exposure to DCA on the pharmacokinetics and metabolism of subsequent doses has not been studied using B6C3F1 mice. Additionally, the influence of prolonged exposure to TCA on its pharmacokinetics has not been previously studied. Thus, the present investigation was designed to characterize the changes in the elimination and metabolism of challenge i.v. and oral doses of DCA and TCA in male B6C3F1 mice after 2 weeks administration of these compounds in drinking water. The metabolism of DCA by liver S9 fractions obtained from control and pre-treated mice was also studied to confirm that changes in clearance are associated with modifications in metabolism of DCA by the liver.

2. Materials and methods

2.1. Chemicals

The dichloroacetic acid ($\approx 99\%$ pure as a free acid) used in dosing solutions and standards was purchased from Fluka Chemical Corp. (Ronkonkoma, NY). Trichloroacetic acid (6.1 N solution, approx. 100% w/v) was obtained from Sigma Chemical Co. (St Louis, MI). Diazomethane, used in the derivatization of the haloacetates, was prepared from 1-methyl-3-nitrosoguanidine (Aldrich Chemical Co. Milwaukee, WI) following Aldrich Technical Information Bulletin Number AL-121. The $[^{14}C_{1,2}]$-DCA and $[^{14}C_{1,2}]$-TCA were obtained from American Radiolabel (St, Louis, MO). The $[^{14}C_{1,2}]$-DCA was obtained in ethanol, where it was found to form an ester upon storage. The ethyl ester was hydrolyzed by mixing one volume of the esterified radiolabel DCA with one volume of 12 N HCl and three volumes of water then hydrolyzed by heating for 14 h at 75°C. Dichloroacetic acid was later purified by HPLC using a PRP-X300 column (Hamilton, Reno, NA) and 0.001 N sulfuric acid as a mobile phase. The radiolabeled TCA was purified in a similar way as DCA. After HPLC purification, radiochemical purity of both DCA and TCA exceeded 99%. Dosing solutions containing 100 mg/ml of the purified dichloroacetic or trichloroacetic acid were prepared by neutralizing to pH ≈ 7 with the addition of NaOH and adjusted to contain between 8 and 12 µCi/ml of the purified $[^{14}C_{1,2}]$-DCA or $[^{14}C_{1,2}]$-TCA.

2.2. In vivo dosing experiments

2.2.1. Animals and treatment

The animal care and experimental protocols were submitted and approved by the Institutional Animal Care and Use Committee (IACUC) of Washington State University and Battelle, Pactfic Northwest National Laboratory. Male B6C3F1 mice (9–10 weeks old) were obtained from Charles Rivers Breeding Laboratories (Raleigh, NC). Mice were administered a single gavage dose of $[^{14}C_{1,2}]$ labeled DCA or TCA (100 mg/kg body weight) and the disposition of the radiolabel, in exhaled breath, urine and feces characterized. Mice were housed separately in glass metabolism cages (Crown Glass Company. Somerville, NJ) and the urine and feces collected for 24 h. Air was drawn through the cages at a rate 200 ml/min by pulling vacuum (Pressure-Vacuum Pump, Model 400-1901, Barnant Company, Barrington, IL). Exhaled CO_2 was collected in two traps connected in series, containing 2 N Sodium hydroxide solution. Aliquots of 0.5 ml of the solution in the traps were taken at 0, 1, 2, 4, 8, 12 and 24 h. Trap solution, urine and feces (1 g in 10 ml of water) samples were analyzed using a scintillation system (Model LS 6500. Beckman Instruments, Inc. Fullerton, CA). In a separate experiment, mice were administered a single gavage dose of $[^{14}C_{1,2}]$ labeled DCA and the blood concentration–time profile of DCA characterized. For this experiment, mice were serially sacrificed at 15, 30 min, 1, 1.5, 2, 3, 4, 8, 16 and 24 h and samples of blood obtained by cardiac puncture.

In additional experiments, the blood concentration–time profiles of DCA and TCA after i.v. administration were determined using non-labeled DCA or TCA. For these experiments, the haloacetates were injected into the lateral tail vein. Ten mice (five pre-treated with 2 g/l of DCA in drinking water for 14 days and five controls) were dosed with 100 mg/kg of DCA to determine the elimination kinetics. Serial blood samples were removed from individual animals via the tail vein (opposite to the vein injected) using heparinized capillary tubes at 10, 20, 30, 45, 60, 120 and 180 min. A similar experiment involving a total of ten mice, four control and six exposed to TCA at 2 g/l in drinking for 14 days, were dosed with 100 mg/kg TCA. Serial blood samples were removed at 20 and 40 min, 1, 2, 3, 8, 12 and 24 h. Between 25 and 35 mg of whole blood was removed at each sampling time. The blood weight was converted to volume assuming a blood density of 1.05 g/ml. The haloacetate pre-treatment dose of 2 g/l was chosen based on its use in cancer bioassays [7]. Table 1 summarizes the in vivo dosing experiments, indicating the challenge dose given and the haloacetate pre-treatment.

2.2.2. In vitro metabolism

Livers were obtained from control (distilled water for 14 days) and pre-treated (2 g/l DCA in drinking water for 14 days) male B6C3F1 mice. Mice were fasted overnight and then sacrificed by CO_2 asphyxiation and the livers immediately perfused in situ with ice-cold 0.9% saline. The perfused livers were homogenized in 50 mM potassium phosphate buffer pH 7.4, 250 mM sucrose and 1 mM ethylene-diamine tetraacetic acid (EDTA). The hepatic tissue was disrupted by six strokes in a glass-Taflon tissue homogenizer with a mechanical drill press. The homogenate was then centrifuged at $9000 \times g$ for 20 min at 4°C. The supernatant (S9 fraction) was retained and stored at -90°C until use. Protein concentration was determined using a coomassie blue reagent (Pierce, IL) with bovine serum albumin use as a standard.

The initial rate of DCA metabolism was determined from the amount of DCA consumed (held to $\leq 10\%$ of initial concentration) after 10 min of incubation with

Table 1
Summary of pre-treatment and challenge dosing experiments using male B6C3F1 mice[a]

Challenge dose		Pre-treatment	
Dosing route	Control	DCA	TCA
DCA (i.v.)	x	x	x
[$^{14}C_{1,2}$]-DCA (gavage)	x	x	n.d.[b]
TCA (i.v.)	x	x	x
[$^{14}C_{1,2}$]-TCA (gavage)	x	n.d.[b]	x

[a] Pre-treated mice were exposed to 2 g/l of DCA or TCA in their drinking water for 14 days. The challenge dose was administered after 16 h fasting and removal of the haloacetate pre-treatment. The number of mice for each pre-treatment and dose was 5 or 6.
[b] n.d. = not determined.

2 mg of S9 protein and 1.5 mM of reduced glutathione in 50 mM potassium phosphate buffer pH 7.4 (total volume of 1 ml). Incubations were stopped by the addition of 50 μl of 50% (v/v) H_2SO_4. Preliminary incubations established that these experimental conditions were linear with respect to incubation time, protein concentration and added GSH. At least six initial concentrations of DCA ranging from 0.002 to 1.16 mM were used. Each DCA concentration was run in triplicate using tissue fractions obtained from individual mice ($n = 5$). The apparent K_M and V_{max} for control and pre-treated mice were obtained using a non-linear regression program (SigmaPlot, Jandel Scientific Software).

2.3. Analytical methods

2.3.1. Gas chromatography

Dichloroacetate and TCA were extracted from 20 to 40 μl of whole blood or 50 μl of S9 incubation solution by mixing with an equal volume of 50% (v/v) H_2SO_4 and 500 μl diethylether. The internal standard (2,2-dichloropropionic acid) was added just before the extraction with diethylether. Next, 300 μl of the organic layer was removed, mixed with 50 μl diazomethane and 400 μl of isooctane. The derivatized haloacetates were analyzed by GC-ECD (DB-WAX column, 30 m × 0.537 mm i.d., 1.0 μm film thickness, J & W Scientific, Folsom, CA). The following GC conditions were used: Carrier gas: He (7.1 ml/min), makeup gas: 95:5% (v/v) of Ar/methane (40 ml/min). Injection port and detector temperature of 225 and 300°C, respectively. Oven temperature: initial temperature 90°C for 6 min, increasing to 160°C (20°C/min) then to 180°C (10°C/min). Retention times for internal standard, TCA and DCA were 5.8, 8.5 and 8.9 min, respectively.

2.3.2. Pharmacokinetic analysis

An iterative, non-linear least squares computer program WIN-NONLIN (Pharsight Corp. Mountain View, CA) was used to fit the individual blood concentration–time profiles (C_b, t) of DCA and TCA after i.v. and gavage administration of DCA. The best statistical fit of the blood concentration–time profile after gavage dosing of DCA in pre-treated mice was a one-compartment model with first-order input, first-order output, and no lag time of absorption, described by the following equation:

$$C_b = [(F/V_d)(K_a^* D)/(K_a - K_e)](e^{-K_e^* t} - e^{-K_a^* t})$$

where D is the dose in μg/kg. The primary estimated parameters were volume/bioavailability (V_d/F), absorption rate (K_a), and elimination rate (K_e) constants. Secondary parameters calculated were area under the blood concentration–time curve, $(AUC) = (K_a F D)/V_d(K_a - K_e)[1/K_e - 1/K_a]$, total body clearance, $Cl_b = K_e(V_d/F)$, terminal half-life ($t_{1/2}$), time of maximum concentration $t_{max} = Ln(K_a/K_e)/(K_a - K_e)$ and maximum concentration, (C_{max}) by substituting t_{max} in the equation for C_b.

The best statistical fit for the mouse i.v. dosing was a one-compartment model with bolus input and first-order output, described by the following equation:

$$C_{\mathrm{b}} = (D/V_{\mathrm{d}}) \, \mathrm{e}^{-ke*t}$$

The model primary estimated parameters were V_{d} and K_{e}. Secondary parameters were area under the curve, $\mathrm{AUC} = D/(K_{\mathrm{e}} V_{\mathrm{d}})$; terminal half-life, $(t_{1/2}) = 0.693/K_{\mathrm{e}}$; $(C_{\mathrm{max}}) = D/V_{\mathrm{d}}$; and total body clearance $(\mathrm{Cl_b}) = K_{\mathrm{e}} V_{\mathrm{d}}$.

The amount of the parent compound (DCA or TCA) excreted in urine for 24 h was quantified to calculate renal clearance $(\mathrm{Cl_r})$ using the equation: $\mathrm{Cl_r} = D_{\mathrm{u}}/[\mathrm{AUC}]_{0 \to 24}$, where D_{u} is the amount of the parent compound collected in urine 24 h post-dosing.

2.3.3. Statistical analysis

Student's *t*-test was used to test for significant differences between pharmacokinetic parameters for control and pre-treated animals. A probability level of ≤ 0.05 (two-tail) was taken as indicating statistically significant differences. The Cochran's *t*-test was used to test for significant differences when parameters showed heterogeneous variances as recommended by Gad and Weil [17].

3. Results

3.1. Pharmacokinetics of i.v. doses of DCA after pre-treatment with DCA or TCA

Mice pre-treated with DCA or TCA at 2 g/l in their drinking water for 2 weeks did not show statistically significant differences in weight gain relative to control animals (data not shown). No alteration in overall water consumption was observed as compared to control mice. The treated mice consumed approximately 392.4 ± 46.3 mg/kg/day of DCA and 404 ± 53.1 mg/kg/day of TCA, calculated based on daily water consumption.

The elimination of an i.v. dose of 100 mg/kg of DCA administered to mice declined as a single exponential process. The DCA concentration–time profile is depicted in Fig. 1 and the derived pharmacokinetic parameters are reported in Table 2. A delay in the elimination of DCA was observed in DCA-pre-treated mice. The DCA-pre-treated animals displayed significant increases in the AUC and terminal half-life $(t_{1/2})$. The total body clearance for the DCA-pre-treated animals was reduced to one-third of that of the control animals, regardless of the route of administration (Table 2). The decreased elimination of DCA from the systemic circulation appears to be due to decreased metabolism since the renal clearance was not reduced with pre-treatment and accounts for a minor fraction of the total clearance. A tendency for renal clearance to increase due to pre-treatment was actually observed, but this was not statistically significant $(P \leq 0.05)$. Both control and pre-treated mice showed equal V_{d} (apparent steady state volume of distribution) indicating that previous exposure to DCA did not affect the extra-vascular distribution (Table 2).

Prior treatment with TCA had minimal effect on the DCA concentration–time profile (Fig. 1). Higher concentrations of DCA on the early points of the curve
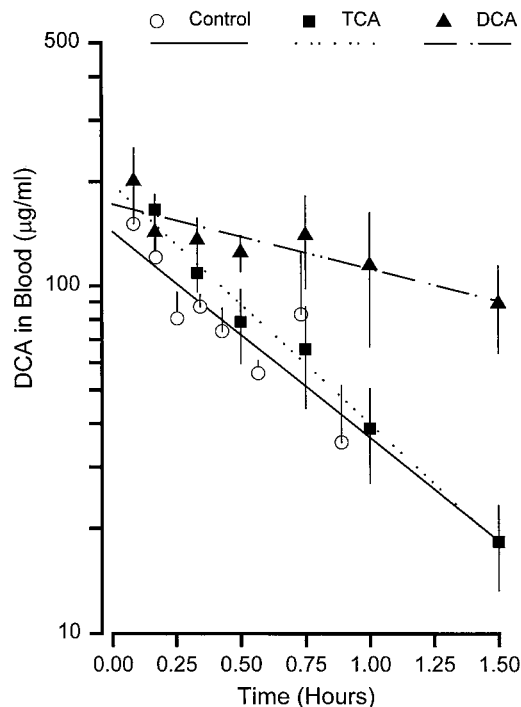
Fig. 1. Blood concentration–time profile from control and pre-treated male B6C3F1 mice after an i.v. (tail vein) dose of 100 mg/kg of DCA. Pre-treated mice were provided with 2 g/l of DCA or TCA in drinking water for 14 days prior to the challenge dose. The i.v. challenge dose was administered after 16 h fasting and removal of DCA or TCA from drinking water. The data shown are means ± S.E.M. of $n = 4$–6 for each treatment group, controls, DCA pre-treated (DCA) and TCA pre-treated (TCA) designated with circles, triangles and squares, respectively.

produced a small, but significant ($P \leq 0.05$) increase in AUC for the TCA pre-treated animals. The terminal half-lives and model predicted maximum DCA concentrations for control and pre-treated mice with TCA were identical (Table 2). The total body clearance for pre-treated mice was decreased to two thirds of that of control animals. The renal clearance was not statistically different compared to controls. Therefore, pre-treatment with TCA had a minimal effect on the disposition of a challenge dose of DCA.

3.2. Pharmacokinetics of gavage doses of DCA

Pre-treatment with DCA substantially altered the pharmacokinetics of subsequent gavage doses of DCA. Fig. 2 depicts the DCA blood concentration–time curve for mice administered a 100 mg/kg dose by gavage. It can be observed that DCA was rapidly absorbed without an apparent lag time. The pharmacokinetic parameters derived from this experiment are also presented in Table 2 to facilitate

comparison with the i.v. kinetics of DCA. DCA concentration could only be quantified at one time point in the naive mice so it was not possible to calculate meaningful pharmacokinetic parameters. In pre-treated mice, a maximum blood concentration of 130 μg/ml of DCA was reached 30 min after dosing. The terminal half-life (≈ 1 h) was very similar to that derived for i.v. dose of 100 mg/kg DCA in pre-treated mice. In naive mice, quantifiable levels of DCA could only be measured at sampling times of 15 and 30 min and were 2.7 and 1.1 μg/ml, respectively. The oral bioavailability or fraction of the dose absorbed (*F*) for DCA was calculated to be 0.86 for the pre-treated mice. The same parameter could not be estimated for the naive mice, but it was clearly much lower, approaching zero.

3.3. Pharmacokinetics of i.v. doses of TCA after pre-treatment with TCA or DCA

The blood concentration–time profiles of a challenge dose of 100 mg/kg of TCA in control and pre-treated male B6C3F1 mice are shown in Fig. 3. No effect of pre-treatment was detected. The TCA blood concentration–time profiles for control, TCA-pre-treated and DCA-pre-treated mice can be described by the same mono-exponential equation used previously. The pharmacokinetic parameters for TCA are presented in Table 3. No statistical significant differences were observed in the pharmacokinetic parameters for control and pre-treated mice.

Table 2
Pharmacokinetic parameters describing the distribution and clearance of an i.v. or gavage dose of 100 mg/kg of DCA to B6C3F1 male mice[a]

Parameter	Route of Administration				
	i.v.	i.v.	i.v.	Gavage dosing	
	Control	DCA pre-treated	TCA pre-treated	Control	DCA pre-treated
V_d (ml/kg)	548 ± 96	534 ± 53	475 ± 26	*[b]	485 ± 57[c]
C_{max} (μg/ml)	200 ± 35	194 ± 21	210 ± 11	3.40 ± 2.3	130 ± 21
AUC (μg/ml/h)	89 ± 12	298 ± 51[d]	122 ± 5[d]	*[b]	255 ± 14[d]
$t_{1/2}$ (h)	0.35 ± 0.1	1.14 ± 0.2[d]	0.40 ± 0.3	*[b]	0.93 ± 0.2[d]
Cl_b (ml/h/kg)	1188 ± 147	387 ± 100[d]	813 ± 37[d]	*[b]	412 ± 98[e]
Cl_r (ml/h/kg)	1.61 ± 0.69	3.13 ± 1.8	2.20 ± 0.61	*[b]	2.30 ± 0.4
Cl_{nr} (ml/h/kg)[f]	1186	384	811	*[b]	410

[a] Control mice received distilled water; pre-treated mice were administered 2g/l of DCA or TCA in drinking water for 14 days. The challenge dose was administered after 16 h fasting and removal of DCA or TCA from drinking water. Parameters reported are means $\pm$ S.E.M. obtained after individual fit of four control, six DCA-pre-treated and four TCA-pre-treated mice.

[b] Unable to calculate.

[c] The volume of distribution of the gavage dose is divided by absolute availability (V_d/F).

[d] Statistically significant different than control, $P \leq 0.05$. AUC and Cl_b were tested using Cochran's *t*-test; other parameters had homogeneous variances.

[e] Total body clearance of the gavage dose is divided by the absolute availability.

[f] Non-renal clearance (Cl_{nr}) was calculated from the difference between the total body (Cl_b) and renal ($Cl_{r,0–24}$) clearances.
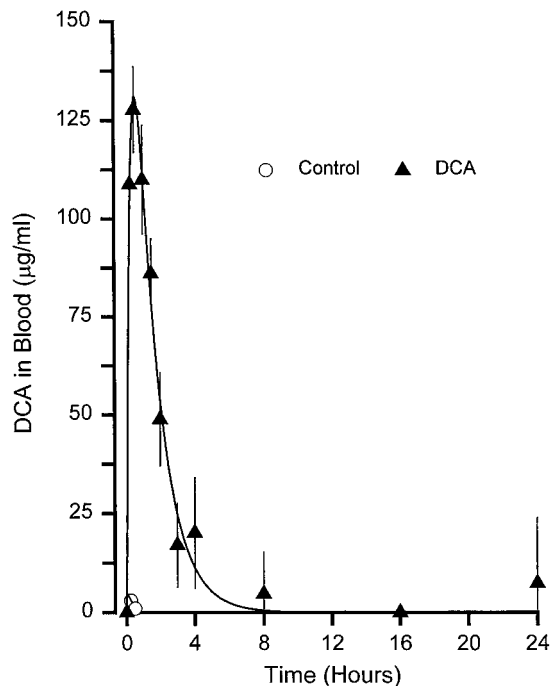
Fig. 2. Effect of pre-treatment in male B6C3F1 mice with 2 g/l of DCA in drinking water on the pharmacokinetic of a gavage dose of 100 mg/kg DCA. The dose was administered after 16 h fasting and removal of DCA from drinking water. Each point is the mean of five animals after serial sacrifice. Controls are indicated by open circles and DCA-pre-treated animals (DCA) by solid triangles. The solid line represents the modelpredicted values.

3.4. Disposition of gavage doses of ^{14}C-DCA and ^{14}C-TCA

Naive and DCA-pre-treated mice were administered ^{14}C radiolabeled DCA by gavage. The ^{14}CO$_2$ in exhaled breath was monitored to test whether the differences observed in the blood concentration–time profiles between naive and DCA-pre-treated mice were due to a modification in the metabolism of DCA. A small increase in urinary excretion of ^{14}C and a slight decrease in CO$_2$ formation were observed in pre-treated mice, but these effects were not statistically significantly different (Fig. 4).

The excretion pattern of an orally administered dose of 100 mg/kg ^{14}C-TCA did not change with pre-treatment (Fig. 5). Approximately 6% of the label administered as TCA was recovered in the exhaled breath in both control and pre-treated mice after 24 h. The production of CO$_2$ for the pre-treated mice was somewhat lower during the first 6 h after dosing. Most of the radiolabel is excreted in urine, $40.8 \pm 12.4\%$ for control and $42.3 \pm 5.4\%$ for the pre-treated mice. A small percentage of the radiolabel was eliminated in feces, $3.7 \pm 1.7\%$ in control and $1.6 \pm 0.6\%$ in the pre-treated mice (Fig. 5 insert). No statistical differences in the amount of

radiolabel eliminated as CO_2 in exhaled air, in urine and feces were observed in the 24-h collection.

3.5. In vitro metabolism of DCA

The rate of DCA metabolism vs. initial DCA concentration is shown in Fig. 6. The apparent V_{max} (mean $\pm$ S.E.M.) for control and pre-treated S9 fractions were 11.3 ± 0.5 and 4.3 ± 0.1 nm/min/mg, respectively. The apparent K_M (mean $\pm$ S.E.M.) for control and DCA-pre-treated mice were 207 ± 30 and 345 ± 65 μM, respectively. These differences were significantly different ($P < 0.05$) as determined from one-way ANOVA. The enzyme kinetic analysis suggested a non-competitive type of inhibition by pre-treatment.
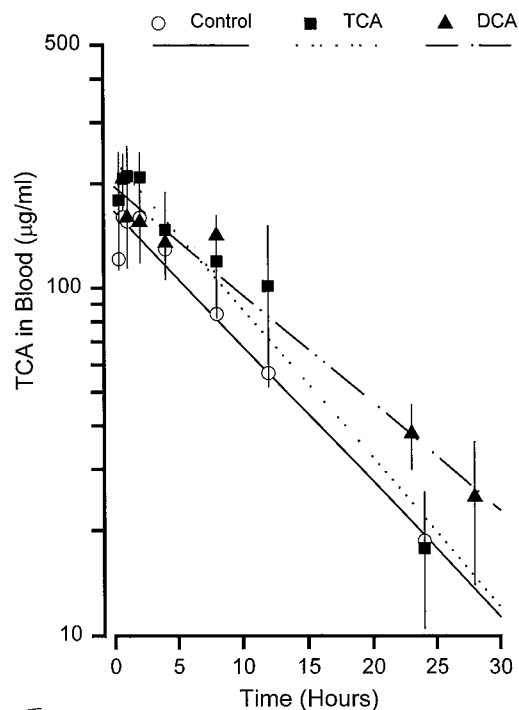

Fig. 3. Blood concentration–time profile from control and pre-treated male B6C3F1 mice after an i.v. (tail vein) dose of 100 mg/kg of TCA. Pre-treated mice were provided with 2 g/l of DCA or TCA in drinking water for 14 days prior to the challenge dose. The i.v. challenge dose was administered after 16 h fasting and removal of DCA or TCA from drinking water. The data shown are means $\pm$ S.E.M. of $n = 4$–6 mice for each treatment group: controls, TCA-pre-treated (TCA) and DCA-pre-treated (DCA) designated with open circles, solid squares and triangles, respectively.

Table 3

Pharmacokinetic parameters describing the distribution and clearance of an i.v. dose of 100 mg/kg of TCA to B6C3F1 male mice[a]

Parameter	Control	TCA pre-treated	DCA pre-treated
V_d (ml/kg)	571 ± 91	483 ± 42	521 ± 15
C_{max} (µg/ml)	179 ± 30	214 ± 17	192 ± 5
AUC (µg/m/h)	2516 ± 289	2964 ± 418	2977 ± 255
$t_{1/2}$ (h)	10.0 ± 2.0	9.40 ± 0.7	10.7 ± 1
Cl_b (ml/h/kg)	40.1 ± 4.6	37.2 ± 5.2	34.0 ± 3.0
$Cl_{r,0 \to 24}$ (ml/h/kg)	28.1 ± 9.1	22.0 ± 3.4	20.2 ± 1.9
Cl_{nr} (ml/h/kg)[b]	12	15	14

[a] Control mice received distilled water; pre-treated mice were administered 2 g/l of TCA or DCA in drinking water for 14 days. The dose was administered after 16 h fasting and removal of DCA or TCA from drinking water. Parameters reported are means $\pm$ S.E.M. obtained after individual fit of four control, six TCA pre-treated and four DCA pre-treated mice.

[b] Non-renal clearance (Cl_{nr}) was calculated from the difference between the total body (Cl_b) and renal ($Cl_{r,0-24}$) clearances.

4. Discussion

The results of this study indicate that prior treatment with DCA significantly affects the disposition and pharmacokinetics of subsequent doses of DCA, but has little affect on the disposition of subsequent doses of TCA. Pre-treatment with TCA has a minimal effect on the metabolism or phamacokinetics of subsequent challenge doses of TCA or DCA in B6C3F1 mice (Fig. 3). These results are consistent with those of Lipscomb et al. [11] who found that DCA co-incubation with TCA had no effect on the degradation of DCA by liver cytosol fractions.

The primary difference between DCA and TCA is the rate at which they are cleared from the systemic circulation. The pharmacokinetic data suggests that the concentration of DCA in blood after an acute dose is largely determined by its rate of metabolism. This contrasts with TCA, where urinary excretion appears to be the primary pathway for elimination. In control mice, the total body clearance of DCA was 30 times higher than that of TCA, largely due to differences in non-renal clearance (Tables 2 and 3). Approximately 50% of a 100 mg/kg gavage dose of DCA is eliminated as CO_2 in 24 h, contrasting with only 6% of an equal dose of TCA. Larson and Bull [16] reported similar percentages of elimination for equal gavage doses of DCA and TCA. The percentages of elimination reported by Green and Prout [14] from a oral dose of 75 mg/kg TCA are also very similar. Thus, non-renal or metabolic clearance makes the highest contribution to the total body clearance of DCA. This is reinforced by the relatively small renal clearance for DCA observed, which was not altered by pre-treatment (Table 2). In contrast, TCA has a higher renal clearance, accounting for about 60% of the total body clearance (Table 3).

Comparison of the non-renal clearance values for mice (Table 2) with those obtained in a pervious study in F344 rats (264 ml/h/kg; [12]) indicate mice have a

significantly higher capacity to metabolize DCA than rats. In vitro metabolism experiments demonstrated that naive mice metabolize DCA approximately two times faster than F344 rats, based on comparisons of intrinsic metabolic clearance (V_{max}/K_M ratio; 3.27 vs. 1.52 ml/h/mg protein) [18]. Mice also appear to be susceptible to autoinhibition of metabolism by DCA as indicated by Fig. 6, but this effect is not as pronounced as that reported for DCA in F344 rats [12]. This is evident in Fig. 4, where DCA pre-treatment had a negligible effect on the $^{14}CO_2$ formation from gavage doses of ^{14}C-DCA, in contrast to findings with F344 rats [12]. Furthermore, the non-renal clearance of DCA in DCA pre-treated mice was still more than 50% greater than in naive F344 rats.

Drugs that are highly metabolized by the liver demonstrate poor systemic availability when given orally. The rapid metabolism of orally administered drugs prior to reaching the systemic circulation is termed first-pass effect [19]. Dichloroacetate clearly has a large first-pass effect in naive mice. Only negligible concentra-
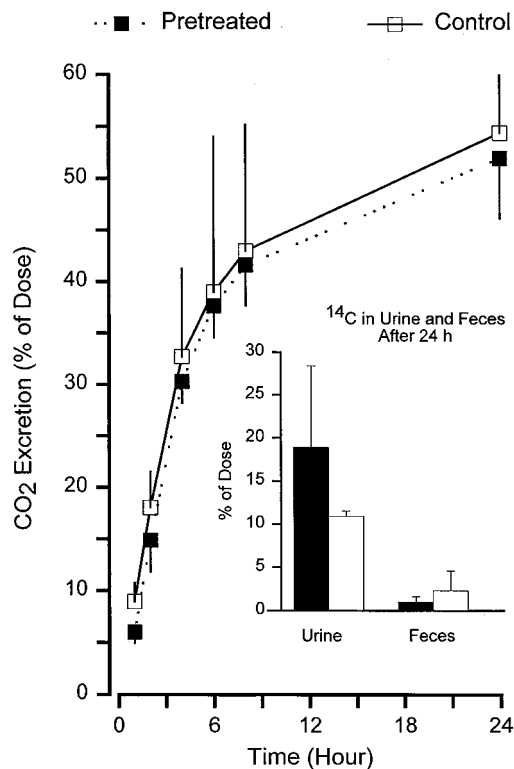

Fig. 4. Effect of pre-treating male B6C3F1 mice with 2 g/l DCA in drinking water for 14 days on the conversion of a gavage dose of 100 mg/kg DCA radiolabeled with [$^{14}C_{1,2}$]-DCA to $^{14}CO_2$. The dose was administered after 16 h fasting and removal DCA from drinking water. Data shown are mean $\pm$ S.E.M. of control mice $n = 5$, represented by open squares and DCA-pre-treated $n = 7$, by solid squares. Insert shows the percent of label excreted in urine and feces (solid bars are DCA pre-treated).
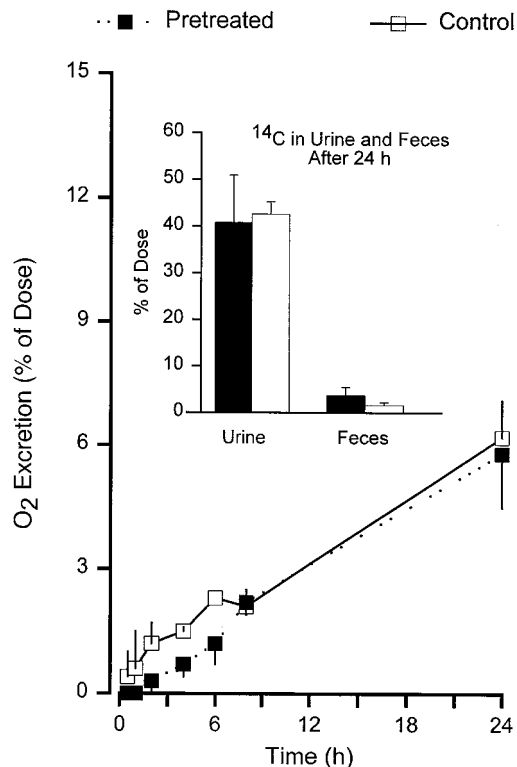
Fig. 5. Effect of pre-treating male B6C3F1 mice with 2 g/l TCA in drinking water for 14 days on the conversion of a gavage dose of 100 mg/kg [$^{14}C_{1,2}$]-TCA radiolabeled to $^{14}CO_2$. The dose was administered after 16 h fasting and removal TCA from drinking water. Control mice, $n = 4$ are represented by open squares and TCA-pre-treated $n = 4$ by solid squares. Insert shows the percent of label excreted in urine and feces (solid bars are TCA pre-treated).

tions ($C_{max} = 3.4$ µg/ml) of DCA could be measured in the blood of naive mice after an oral dose of 100 mg/kg. However, in mice with reduced DCA metabolism caused by DCA pre-treatment, much higher blood levels were observed ($C_{max} = 130$ µg/ml) and the oral bioavailability increased to 86% (based on AUC ratios, Table 2). These differences arise from what appear to be comparatively modest changes in non-renal clearance (approximately 3-fold, Table 2). In this case, the peak blood concentrations from the same dose (100 mg/kg) administered to the pre-treated-mouse increased blood levels by almost 50-fold. A similar effect probably accounts for the large differences in peak blood levels observed in mice exposed to DCA at relatively small differences in DCA concentrations in drinking water. A recent study found that increasing DCA concentration in drinking water from 0.5 to 2 g/l increased peak blood concentrations from 3.5 to about 300–400 µM [20].

In summary, the metabolism of DCA in mice is susceptible to inhibition by prior treatment with DCA. However, the impact on clearance is less marked in mice than
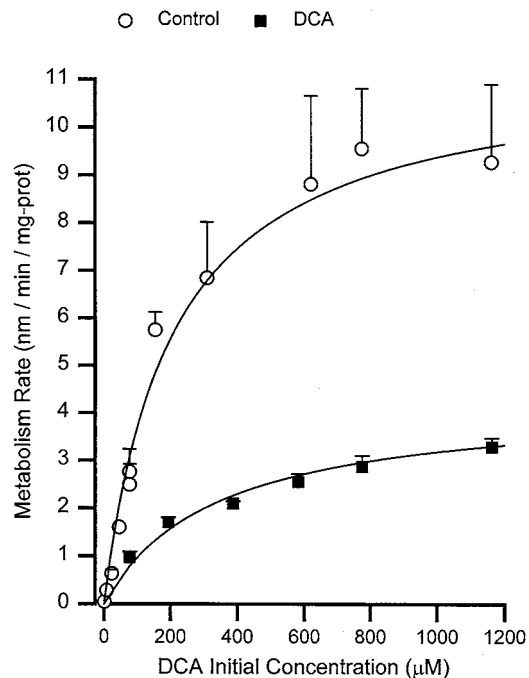
Fig. 6. Initial rate of DCA disappearance, using liver S9 fractions obtained from liver of control, $n = 5$, designated by open circles and DCA-pre-treated mice (DCA), $n = 5$ by solid squares. Pre-treated mice were administered with 2 g/l DCA in drinking water for 14 days. The livers of control and pre-treated mice were excised after 16 h fasting and removal of DCA from drinking water. Solid lines are a representative non-linear fit of the observed data to the Michaelis–Menten equation.

in rats [12]. Our results when combined with those of previous studies [11–13], indicate that prolonged exposure to DCA in rats and mice primarily inhibits a GSH dependent pathway that exists in the cytosolic fraction of liver homogenates. Recently, the metabolism of DCA has been described by Tong et al. [21] to involve a glutathione-S transferase of the Zeta class. However, further research is needed to determine whether this enzyme is the molecular target for DCA inhibition.

Acknowledgements

References

[1] P.C. Singer, Disinfection by-products: From source to tap, in: Disinfection by products in Drinking Water: Critical Issues in Health Effects Research. Workshop Report. International Life Science Institute Health and Environmental Sciences Institute, 1995.

[2] J.J. Westrick, J.W. Mello, R.F. Thomas, The ground water supply survey, J. Am. Water Works Assoc. 75 (1984) 52.

[3] National Cancer Institute. Carcinogenesis bioassay of trichloroethylene. CAS No. 79-01-6. DHEW. Pub. No. (NIH) 76-802, 1976.

[4] C. Maltoni, G. Lefemine, G. Cotti, Experimental research on trichloroethylene carcinogenesis, in: C. Maltoni, M.A. Mehlman (Eds.), Archives of Research on Industrial Carcinogenesis, Princeton Scientific, Princeton, NJ, 1986.

[5] National Toxicology Program. Toxicology and carcinogenesis studies of trichloroethylene in four strains of rats. CAS No. 79-01-6, NTPTR 273. Research Triangle Park, NC. U.S. Department of Health and Human Services, Public Health Services, National Institute of Health, 1988.

[6] National Toxicology Program. Draft technical report on the toxicology and carcinogenesis studies of chlorinated and chloraminated water in F344/N rats and B6C3F1 mice. Research Triangle Park, NC, 1990.

[7] R.J. Bull, I.M. Sanchez, M.A. Nelson, J.L. Larson, A.J. Lansing, Liver tumor induction in B6C3F1 mice by dichloroacetate and trichloroacetate, Toxicology 63 (1990) 341–359.

[8] A.B. DeAngelo, F.B. Daniel, A.J. Stober, G.R. Olson, The carcinogenicity of dichloroacetic acid in the male B6C3F1 mouse, Fund. Appl. Toxicol. 16 (1991) 337–347.

[9] S.L. Herren-Freund, M.A. Pereira, M.D. Khoury, G. Olsen, The carcinogenicity of trichloroethylene and its metabolises, trichloroacetic and dichloroacetic acid, in mouse liver, Toxicol. Appl. Pharmacol. 90 (1987) 183–189.

[10] S.H. Curry, A. Lorenz, P. Chu, M. Limacher, P.W. Stacpoole, Disposition and pharmacodynamics of dichloroacetate (DCA) and oxalate following oral DCA doses, Biopharm. Drug Dis. 12 (1991) 375–390.

[11] J.C. Lipscomb, D.A. Mahale, W.T. Brashear, H.A. Barton, Dichloroacetic acid: Metabolism in the cytosol, Drug Metab. Dis. 23 (1995) 1203–1205.

[12] A. Gonzalez-Leon, I.R. Schultz, G. Xu, J.R. Bull, Pharmacokinetics and metabolism of dichloroacetate in the F344 rat after prior administration in drinking water, Toxicol. Appl. Pharmacol. 146 (1997) 189–195.

[13] M.O. James, R. Cornett, Z. Yan, G.N. Henderson, P.W. Stacpoole, Glutathione-dependent conversion to glyoxylate, a major pathway of dichloroacetate biotransformation in hepatic cytosol from humans and rats, is reduced in dichloroacetate-treated rats, Drug Metab. Dis. 25 (1997) 1223–1227.

[14] T Green, M.S. Prout, Species differences in response to trichloroethylene. II. Biotransformation in rats and mice, Toxicol. Appl. Pharmacol. 79 (1985) 401–411.

[15] J.W. Fisher, T.A. Whittaker, D.H. Taylor, H.J. Clewell III, M.E. Andersen, Physiologically-based pharmacokinetic modeling in the pregnant: A multiroute exposure model for trichloroethylene and its metabolise trichloroacetic acid, Toxicol. Appl. Pharmacol. 99 (1989) 395–414.

[16] J.L. Larson, R.J. Bull, Metabolism and lipoperoxidative activity of trichloroacetate and dichloroacetate in rats and mice, Toxicol. Appl. Pharmacol. 115 (1992) 268–277.

[17] S.C. Gad, C.S. Weil, Statistic for toxicologists, in: A.W. Hayes (Ed.), Principles and Methods in Toxicology, Raven Press, New York, NY, 1994, pp. 221–274.

[18] A. Gonzalez-Leon, J.L. Merdink, I.R. Scultz, R.J. Bull, Dichloroacetate auto-inhibits its degradation in the cytosol, Toxicol. Sci. 42 (1-S) (1998) 86.

[19] L. Shargel, A.B.C. Yu, Hepatic elimination of drugs, in: Applied Biopharmaceutics and Pharmacokinetics, 3rd ed, Appleton and Lange, Norwalk, CT, 1993, p. 315.

[20] J. Kato-Weinstein, M.K. Lingohr, G.A. Orner, B.D Thrall, R.J. Bull, Effects of dichloroacete on glycogen metabolism in B6C3F1 mice, Toxicology 130 (1998) 141–154.

[21] Z. Tong, P.G. Board, M.W. Anders, Glutathione transferase Zeta catalyses the oxygenation of the carcinogen dichloroacetic acid to glyoxylic acid, Biochem. J. 331 (1998) 371–374.